The Use of Magnetotactic Bacteria to Remove Phosphorus from Eutrophic Conditions

An Honors Undergraduate Thesis

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By

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Abstract

Eutrophication or excess nutrients in rivers and lakes is a problem in the Midwest commonly caused by high levels of phosphorus in runoff from agricultural land. Magnetotactic bacteria (MTB), a magnetite-containing microorganism found in aquatic ecosystems, may contain intracellular inclusions of phosphorus. This study will test how effective MTB is at removing high concentrations of phosphorus from eutrophic conditions. The hypothesis for this project is that MTB will have some capability to remove phosphorus from their water environments, offering a microbiological solution in places where eutrophication occurs. Methods include growing the type strain of MTB, *Magnetospirillum magneticum*, AMB-1, in media spiked with concentrations of phosphorus at 0.01 mg/L, 0.025 mg/L, and 0.06 mg/L. These concentrations were selected to mimic, respectively, Canada's target level of phosphorus for Lake Erie, the US Environmental Protection Agency's target level of phosphorus in lakes, and the peak phosphorus levels found in the western Lake Erie basin in 2010. A colorimetric analysis was used to measure phosphorus in solution at different time points. A centrifuge was used to separate the cells from the media. Results indicate that when phosphorus-containing media is inoculated, concentrations of phosphorus decrease in the media after two days. Samples with higher concentrations of phosphorus experience more rapid decreases in solution phase phosphorus. Phosphorus was recovered from the cell pellet, indicating phosphorus was removed and stored in AMB-1 cells. Results indicate this technology may hold some promise for limiting eutrophic conditions such as those that occur in northwest Ohio and Lake Erie.

Keywords: eutrophication, magnetotactic-bacteria, modeling, data analysis

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Introduction

Eutrophication or excess nutrients in rivers or lakes is a problem in the Midwest commonly caused by high levels of phosphorus in runoff from agricultural land. For example, Lake Erie is eutrophic because agricultural runoff, nutrients that flow into the lake, contains phosphorus (Rea et al., 2015). Magnetotactic bacteria (MTB), a magnet-containing microorganism found in aquatic ecosystems, have been found to contain intracellular inclusions of phosphorus. In this study, I will test if magnetotactic bacteria can be used to remove phosphorus from eutrophic conditions. Because these bacteria are naturally magnetic, phosphorus-enriched cells can be easily removed from the water by using a magnet. This process could offer a microbiological solution in places like Lake Erie, where eutrophication occurs. The phosphorus-enriched cells can then be removed with a simple magnet and disposed of or reused in a more environmentally-friendly fashion (e.g., used as fertilizer). The goals of the project are to (1) learn how to collect freshwater samples and isolate MTB, (2) grow *Magnetospirillum magneticum* AMB-1, a type strain of MTB that I will use in my experiments, with different levels of phosphorous, (3) use a colorimetric assay to measure the amount of phosphorous lost from the samples, (4) present my findings at both the College of Food, Agriculture, Environmental Science research forum and the Denman research forum. The purpose of this thesis is to test if MTB could be used to remove phosphorus from eutrophic conditions and use a literature review to understand the additional considerations of using this technology in places where eutrophication occurs, such as Lake Erie.

Background Information

Literature Review

Magnetotactic Bacteria

MTB is a prokaryotic, magnetite-containing microorganism found in aquatic ecosystems. The organism is ubiquitous to both freshwater and marine environments. It is typically found in the area between the water and sediment interface, as the organism prefers environments with little to no oxygen present (Bazylinski et al., 1995). Due to the environment where MTB is found, it is thought to be one of the first organisms to inhabit the Earth (Chen et al., 2010). MTB use magnetotaxis to orientate themselves according to the geomagnetic field of the Earth (Chen et al., 2010). MTB also have a flagella, which can rotate clockwise or counterclockwise depending on how much oxygen is present in their water environment and if they are in the Northern or Southern Hemisphere, to propel themselves forward or backward (Chen et al., 2010).

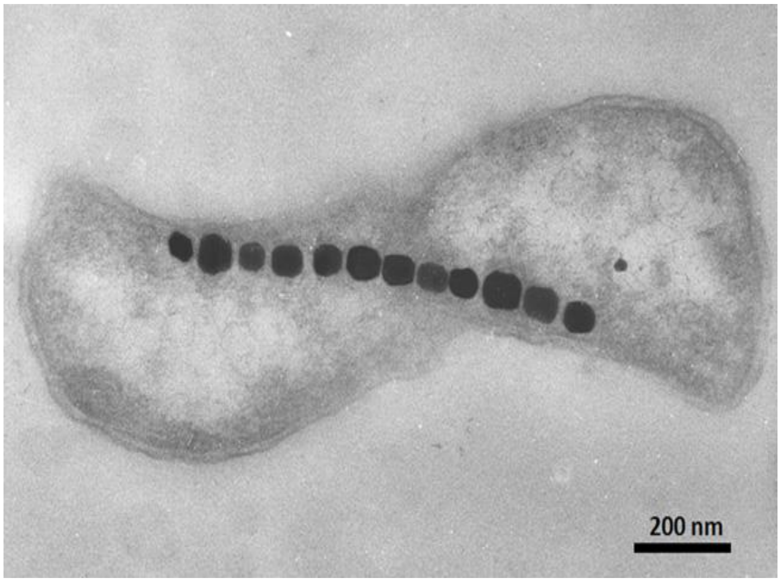

Figure 1. Depicts MTB under a TEM. The line of circular structures represents the magnetite found within MTB. Using MTB magnetite, these organisms can be moved magnetically. The lighter gray coloring within MTB represents where MTB intracellular inclusions are typically present. *Photo courtesy: L. Chen, D. Bazylinski & B. Lower*

Due to the unique natural of MTB, these bacterial cells have been of great interest for many applications from environmental magnetism, bioremineralization, paleomagnetism, and more (Yan et al., 2012). Most of the interest has been focused on manipulating these organisms in the medical and biological sciences, but there are still many other applications for MTB. One potential application is using MTB in eutrophic waters to remove phosphorus. In previous research, MTB organisms have been found to contain intracellular inclusions of phosphorus (Oestreicher et al., 2011). This indicates MTB do have the capability to remove certain nutrients from an environment. Additionally, other research has been able to successfully trap motile MTB in water solutions with magnetic recording heads (i.e. a large magnet placed in solution), indicating MTB can be removed from a water environment (Krichevsky et al., 2007). However, there is a gap of knowledge about how and if this technology could be applied in the context of a eutrophic water system, such as Lake Erie.

This research project and thesis will help improve the existing knowledge about MTB in water environments and eutrophic conditions. Using MTB in remove phosphorus in eutrophic waters has the potential to offer a biological solution in places where eutrophication occurs and could have the ability to improve systems like Lake Erie. In this study, I will examine if MTB have the ability to remove phosphorus from eutrophic waters.

Lake Erie

<u>Cause for Concern</u>

Lake Erie is a complicated system that has been struggling in recent years. The system has and continues to be threatened with eutrophic conditions know colloquially as algal blooms. These blooms are often seen as a green colored layer covering the surface of Lake Erie and can become so large they can be monitored and tracked from outer space (Figure 2). In certain conditions, these blooms can turn into harmful algal blooms (HABs), when Microsystis, a freshwater cyanobacterial species that produces a hepatotoxin called Microsystin, forms. This is not only an environmental concern, but also as public health concern. Due to the hepatotoxin nature of Microsystin, water can become undrinkable and pets or livestock can be killed from the toxin. One of the most notable examples of these effects happened during the Toledo drinking water crisis of 2014, where water was undrinkable for three days due to high amounts of Microsystis present in the city's drinking water supply (Jetoo et al., 2015). This crisis affected over 300,000 in the state of Ohio and demonstrates how crucial it is to finding solutions to reduce the eutrophic conditions in Lake Erie.

Figure 2. Lake Erie's western basin is one example of where eutrophication occurs. Satellite images can even show the span of the blooms. *Photo courtesy: NASA*

Contributing Factors

Briefly, there are variety of factors that attribute to the reoccurring eutrophic conditions present in Lake Erie. To begin, Lake Erie is the shallowest of the Great Lakes and has an average depth of approximately twenty-five meters in the western basin, where most of the blooms occur. Being a shallow environment, Lake Erie is already prone to more eutrophic conditions (Watson et al., 2016). Additionally, Lake Erie is surrounded by agricultural land, which creates a substantial amount of fertilizer and nutrient runoff going directly into Erie (Bridgeman et al., 2012). The Maumee River, which is the largest tributary river to Lake Erie, also primarily contains all agriculture runoff draining into its watershed, which contributes to the eutrophication problem (Baker et al., 2014; Bridgeman et al., 2012). An additional consideration can also be given to meteorological trends. Should the weather be warm with little to no wind present on Lake Erie for a duration of time, a bloom can also form (Michalak et al., 2013). This happens because when the lake is not mixed, stagnant water conditions form, which creates an optimal growing environment for cyanobacterial species (Michalak et al., 2013). A final consideration can also be given to the rising effects of climate change. As temperatures continue to rise in Lake Erie, an even more optimal growing condition for cyanobacteria is created (Watson et al., 2016). Combining these effects all together equate to multiple variables contributing to the eutrophication problem in Lake Erie.

Food Webs and MTB

It is also important to understand where MTB fits within an aquatic environment, such as Lake Erie. However, little is known about the ecological role of MTB in aquatic and sedimentary environments. The ability of MTB to remove elements, like phosphorus, from a water system does suggest that MTB may play an important role in various biogeochemical cycles in water environments. For example, MTB can be consumed by its main predator (i.e. protozoa), which leads to phosphorus or other elements reentering the food chain (Lefèvre et al., 2011). On the other hand, when MTB die, part of or the entire magnetofossils can be dissolved, which releases phosphorus or other elements back into the environment.

In terms of food webs and trophic levels, MTB are primarily consumed by protozoa (Lefèvre et al., 2011). Common protozoa found in Lake Erie are *Sarcomastigophora, Cilophora*, and *Rhizopoda* species. These species can be categorized as heterotrophs and autotrophs. Many magnetite-producing MTB are chemoorganoheterotrophic, while other MTB species are facultatively chemolithoautotrophic or obligately chemolithoautotrophic (Lefèvre et al., 2011; Bazylinski et al., 2013). The diversity of MTB is extremely vast, but based on previous research, a majority of the MTB present in Lake Erie are likely chemoorganoheterotrophic (Lefevre & Bazylinski, 2013). MTB are also primarily mesophiles, as they are typically restricted to habitats with a neutral pH (Lefevre & Bazylinski, 2013). Understanding where MTB fits in the complicated system of Lake Erie is important for discussing the application of this technology to eutrophic waters.

Methods

For MTB collection and phosphorus analysis, samples of water and sediment were collected at the Olentangy Wetland Research Park during the spring and winter when phosphorus levels could be high and low, respectively, to get a thorough understanding of where MTB naturally occurs and how to isolate it in nature (Dolman et al., 2016). For the lab work involving MTB and phosphorus, *Magnetospirillum magneticum* AMB-1 was cultured using ATCC medium 1653 in Schott bottles (Oestreicher et al., 2016). This type strain will be used because it is a standard MTB used by many researchers and can be cultured more easily than many natural isolates.

Procedural wise, sample collection and preservation followed standard methods outlined in Methods for chemical analysis of water and wastes (1979). Briefly, samples were stored at 4° C in plastic or glass and examined for phosphorus within 28 days of collection (Eaton &

Franson, 1995). For microbiological analysis, the hanging drop method was used to confirm MTB are present (Sorty & Shaikh, 2014). MTB was isolated according to Oestreicher et al by placing a simple bar magnet along the outside of a sample container at the sediment water interface (2012).

M. magneticum AMB-1 was grown at 28° until they reach the exponential or midlog phase as determined by measuring the optical density at 565 nm in a spectrophotometer. Varying phosphorus concentrations were then added to the bacteria solutions. One series of experiments used a low concentration of 0.1 mg/L of phosphorus. In another series of experiments with medium phosphorus concentrations, I will add 0.25 mg/L of phosphorus to the MTB solution. In a series with high phosphorus concentration, I added 0.06 mg/L of phosphorus. These concentrations were selected to mimic, respectively, Canada's target level of phosphorus for Lake Erie, the US Environmental Protection Agency's target level of phosphorus in lakes, and the peak phosphorus levels found in the western Lake Erie basin in 2010. A control of dead cells was also used to compare results and ensure MTB was actively removing phosphorus.

Approximately within an hour of adding phosphorus, I centrifuged the solutions to remove bacteria. A portion of the supernatant (i.e. water minus the MTB) was then analyzed for phosphorus using the ascorbic acid procedure (Dupas et al., 2015). An absorbency reading for supernatants with each phosphorus concentration was obtained using a spectrophotometer. I was then able to determine the amount of phosphorus removed by the cells by subtracting the starting concentration (0.01 mg/L for example) from the remaining concentration in the supernatant.

Results

Results found concentrations of phosphorus decreased in the media rapidly when phosphorus containing media is inoculated. Phosphorus was recovered from the supernatant in each phosphorus concentration (Figure 3 & Figure 4). Samples with higher concentrations of phosphorus were found to experience more rapid decreases in solution phase phosphorus.

Between low final phosphorus concentrations of 0.01 mg/L and high final phosphorus concentrations of 0.06 mg/L, AMB-1 significantly removed phosphorus ($t=-2.63$, $p<0.05$, $n=6$) (Table 1). Between low final phosphorus concentrations of 0.01 mg/L and high final phosphorus concentrations of 0.06 mg/L, AMB-1 did not significantly removed phosphorus ($t=-2.55$, $p>0.05$, $n=6$) (Table 2). When comparing between medium final phosphorus concentrations of 0.025 mg/L and high final phosphorus concentrations of 0.06 mg/L, AMB-1 did not significantly removed phosphorus ($t=-2.31$, $p>0.05$, $n=6$) (Table 3). Microbiologically, this could be attributed to an overabundance of phosphorus being present that the MTB cells died and were therefore, not able to remove phosphorus from the environment. Further evaluation using a transmission electron microscope (TEM) could help determine if this were the case. To ensure differences in the low and high phosphorus concentrations were truly significant, a control of dead cells was compared statistically and no significance was found. Results were all analyzed using a two-tailed t-test to test for difference between the various phosphorus concentrations in the supernatant.

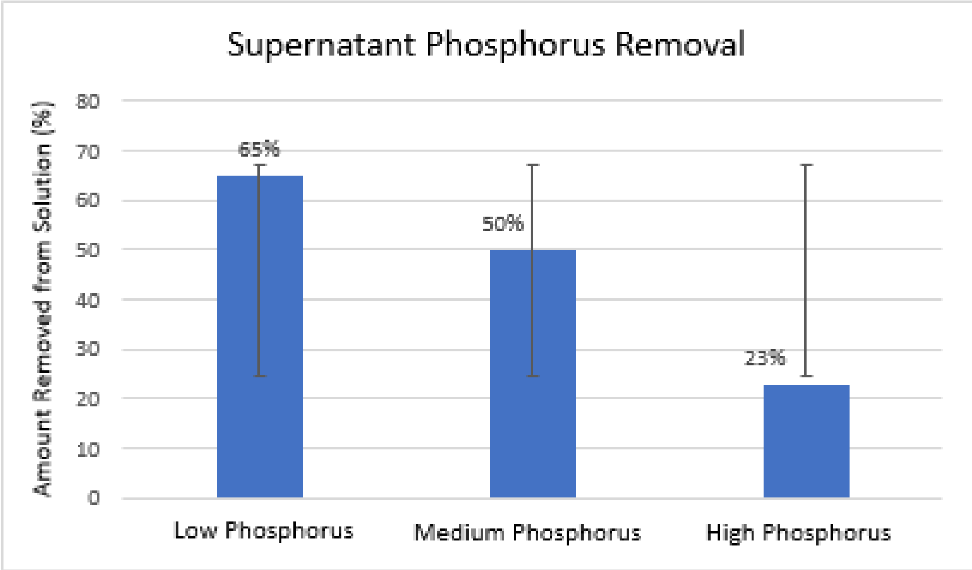

Figure 3. This represents the amount of phosphorus removed, as a percentage, from the supernatant for each of the initial phosphorus concentrations. Standard deviation is also represented for the low, medium, and high phosphorus concentrations.

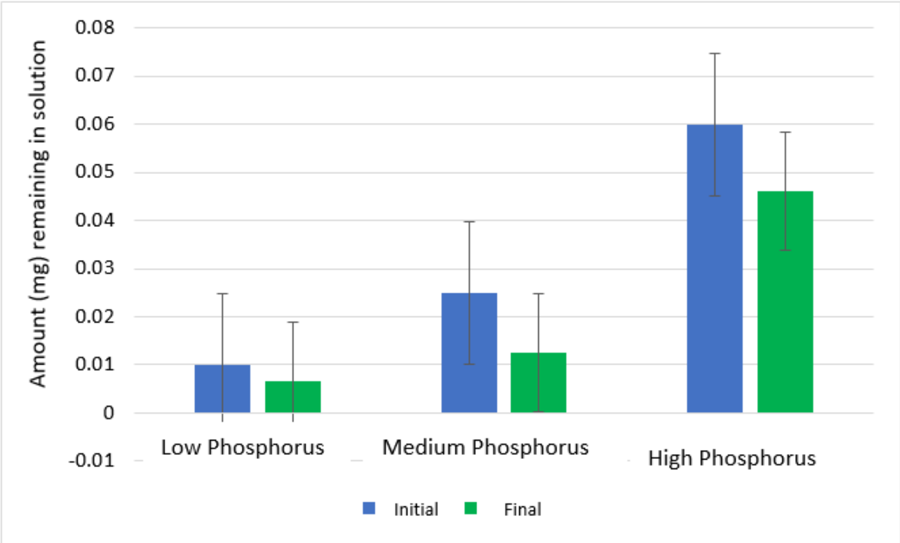

Figure 4. This represents the amount of phosphorus removed, in milligrams, from the supernatant for each of the initial and final phosphorus concentrations. Standard deviation is also represented for the low, medium, and high phosphorus concentrations.

Table 1. Represents the t-test results between the low (variable one) and high (variable two) final concentrations of phosphorus at in the supernatant.

	Variable 1	*Variable 2*
Mean	0.0205	0.05
Variance	0.0004531	0.000301
Observations	6	6
Hypothesized Mean Difference	0	
df	10	
t Stat	-2.6317266	
P(T<=t) one-tail	0.012543849	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	0.025087699	
t Critical two-tail	2.228138852	

Table 2. Represents the t-test results between the low (variable one) and medium (variable two) final concentrations of phosphorus at in the supernatant.

	Variable 1	*Variable 2*
Mean	0.021	0.033
Variance	0.0004531	0.000143
Observations	6	6
Hypothesized Mean Difference	0	
df	10	
t Stat	-2.5515762	
P(T<=t) one-tail	0.245835681	
t Critical one-tail	1.835824611	
P(T<=t) two-tail	0.0235861	
t Critical two-tail	2.2343568	

Table 3. Represents the t-test results between the medium (variable one) and high (variable two) final concentrations of phosphorus at in the supernatant.

	Variable 1	*Variable 2*
Mean	0.030	0.051
Variance	0.000112	0.000371
Observations	6	6
Hypothesized Mean Difference	0	
df	10	
t Stat	-2.31116	
P(T<=t) one-tail	0.077099	
t Critical one-tail	1.713582	
P(T<=t) two-tail	0.022159	
t Critical two-tail	2.354357	

Discussion

Results suggest MTB have some ability to remove phosphorus when comparing the low and high final phosphorus concentrations (0.01 mg/L and 0.06 mg/L). Since these concentrations were modeled after real target limits for Lake Erie, the technology does hold some promise for removing phosphorus from eutrophic waters. However, it is important to consider the entire system of Lake Erie. To implement this technology in a natural system, additional replications and the use of a TEM are essential to understand first and to visually determine if phosphorus is in MTB. Additionally, natural MTB would need to be used to understand if MTB already occurring in water environments would have the same ability to remove phosphorus. It is important to proceed with caution before this technology is applied to any natural system.

While the comparison between the low and high final phosphorus concentrations indicate successful results, the comparisons between the low (0.01 mg/L) and medium (0.025 mg/L), and medium (0.025 mg/L) and high (0.06 mg/L) final concentrations of phosphorus do not. I hypothesize MTB could not significantly remove phosphorus in the latter comparisons because there was simply too much present. The concentration of 0.025 and 0.06 mg/L can be a toxic phosphorus limit for a variety of microorganisms and aquatic life. MTB only have the ability to store so much phosphorus, and past that limit it could be detrimental. This could be of concern should there be extremely high phosphorus concentrations present in a water system. This technology may therefore, not be as promising when phosphorus levels reach a beyond reasonable eutrophic level. However, it is important to conduct further replications to understand the high phosphorus concentration fully.

Technology Application

To apply this technology to a system like Lake Erie, natural MTB could be dispersed into a eutrophic body of water or the MTB already occurring in a water body could be used. This could be done growing large portions of MTB in a laboratory and adding them to a water system or using the MTB naturally present in Lake Erie to remove phosphorus. Since MTB occur in water systems naturally around the world and in Lake Erie, for example, a new bacterial species would not have to introduced to any water system. After a period of time, a large magnet could be used to remove the MTB, which will have “soaked up” all of the excess phosphorus from the water environment. These large magnets could be placed on the back of a boating vessel, for example. By removing MTB, phosphorus could also be removed from a water system entirely.

Once MTB is removed from the water environment it could also be recycled and used in fertilizer applications. For example, the MTB, containing phosphorus, could be repurposed on agricultural land instead of adding more phosphorus in fertilizer applications. Recycling the phosphorus would decrease the overall amount of phosphorus runoff flowing into eutrophic waters. This could be especially helpful in areas, such as the Maumee River, where primarily all the runoff comes from agricultural land.

Future Research Direction

To thoroughly understand the potential of this technology, I would recommend a transmission electron microscope (TEM) be used to confirm phosphorous is inside of the MTB cells in the form of intracellular inclusions, which are subcellular bodies found within the bacterium. Due to the financial and time barriers of this project, a TEM was not used in the analysis of this project. Using a TEM would add a key visual as to specifically where phosphorus and other nutrients are being stored. From here, a TEM could also be used to understand how MTB stores these elements. For example, a visual can show if nutrients are stored in intracellular inclusion of MTB and if it is metabolized.

Another way to help determine if MTB are significantly removing phosphorus in future research, could be to compare other bacteria species that have the ability to remove phosphorus, such as *Escherichia coli (E. coli)* with the same methods and analysis (Yang et al., 2016). *E. coli* could be used because it is a standard bacterium used by microbiologists. This could be used to support if the sixty-five percent removed from the low phosphorus concentration, for example, is truly a large amount of phosphorus removed from MTB.

Future studies could also measure out the same number of cells using a bacteria growth curve. Cells could be removed, diluted approximately six times, and plated on a petri dish to count the amount the colony forming units present in each milliliter. Knowing the same number of cells are present in each treatment would help normalize the starting concentrations and overall amount of cells present in each phosphorus treatment.

Conclusion

The purpose of this thesis was to test if magnetotactic bacteria could be used to remove phosphorus from eutrophic conditions and use a literature review to understand the additional considerations of using this technology in places where eutrophication occurs, such as Lake Erie. Utilizing this technology could offer an exciting and innovative microbiological solution to remove phosphorus. While this technology may hold some promise for places where eutrophication occurs, more research is still needed to determine how effective this technology is.

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